



UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
"CAROL DAVILA" din BUCUREȘTI



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"CAROL DAVILA", BUCHAREST
DOCTORAL SCHOOL
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***THE ROLE OF COLLAGEN IN INCISIONAL HERNIA
FORMATION AND RECURRENCE***
DOCTORAL THESIS ABSTRACT

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Dedication

This doctoral thesis is the result of a long academic journey, which would not have been possible without the guidance and support of remarkable individuals.

I would like to express my deepest gratitude to my supervisor, Prof. Dr. Traean Burcoș, for his academic rigor, precise guidance, constant encouragement, and invaluable advice, all of which have significantly contributed to the completion of this study.

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The example set by these remarkable professionals will continue to be a source of inspiration throughout my entire career.

"Mens, manus, et cor!"

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Introduction

Surgical advances, particularly in abdominal surgery, have significantly improved patient outcomes. However, incisional hernias remain a major postoperative challenge due to their high recurrence rates, imposing a burden on health systems. Despite advances in surgical techniques, recurrence persists, which has spurred research into biological and mechanical factors. Collagen, the main structural protein of connective tissue, whose synthesis and remodeling are essential for maintaining the integrity of the abdominal wall after surgery [1,2], is at the center of these issues.

Hernia recurrence extends beyond mechanical failure, involving complex biological processes that govern wound healing. Collagen, essential for tensile strength and structural support, plays a critical role in this context [3,4]. A disturbed balance between collagen types, particularly a low ratio of type I/III, leads to weaker scar tissue, increasing susceptibility to herniation [3,4].

This PhD thesis aims to explore the complex relationship between collagen metabolism and incisional hernia recurrence. By synthesizing existing literature and conducting empirical research, it investigates the molecular mechanisms underlying the role of collagen in this surgical complication. The study examines collagen synthesis and degradation pathways and how surgical interventions affect collagen integrity in scar tissue.

Beyond hernia management, this research contributes to a broader understanding of injury healing, potentially shaping personalized surgical strategies and improvements in postoperative care. By furthering knowledge of collagen dynamics, it aims to refine surgical methodologies and advance innovations in patient care.

Thus, the thesis "The role of collagen in incisional hernia recurrence" aims to be an essential academic reference that sheds light on the complexity of hernia recurrence through an in-depth examination of the role of collagen. Its findings may significantly improve surgical outcomes, paving the way for more effective prevention and management of incisional hernias. To achieve this, we conducted a rigorously designed prospective study with a statistically significant randomized case series.

GENERAL PART - 1. Current state of knowledge

1.1. Importance of the problem

Recurrent incisional hernia represents a significant challenge in abdominal surgery, with a considerable impact on patient morbidity and healthcare system costs. With a prevalence of up to 20% in the postoperative period [5,6], this condition complicates surgical treatment, increasing the risk of failure and necessitating repeated interventions [6,7]. Factors involved in recurrence include the patient's biological healing process, the surgical technique used and the type of material used. In recent decades, the introduction of tension-free repair and synthetic mesh has been a significant advance, reducing recurrence rates [5,6,7]. However, the success of the procedure remains dependent on the choice of mesh, its correct positioning and the experience of the surgeon [7,8]. In addition to these technical factors, patient biology plays a key role in the risk of recurrence. Studies show that an altered ratio of type I collagen to type III collagen is associated with increased connective tissue fragility, predisposing patients to recurrence [2]. Also, factors such as obesity, smoking and comorbidities worsen the prognosis and influence the long-term success of surgery. Despite advances in surgical techniques and the use of prosthetic materials, the wide variations in recurrence rates suggest the need for standardized therapeutic strategies integrated into evidence-based clinical guidelines [7,8].

1.2. Abdominal wall anatomy

The abdominal wall is a complex, multilayered musculoskeletal structure that contributes to the maintenance of intra-abdominal pressure, respiration, posture and protection of internal organs [9,10].

Abdominal muscles, such as the transversus abdominis, obliques and rectus abdominis, play an essential role in stabilizing the trunk and regulating intra-abdominal pressure [11]. A fundamental component of the abdominal wall architecture is the linea alba, a midline fibrous structure that distributes mechanical forces. When this structure is weakened, herniation is favored [12].

Vascularization of the abdominal wall is provided by the epigastric, intercostal and iliac arteries and is crucial for postoperative healing [9,10]. In addition, innervation of the abdominal

wall, controlled by the thoracoabdominal, subcostal and iliohypogastric nerves, influences muscle contraction and pain perception [9,10].

1.3. Biomechanical properties of the abdominal wall

The strength of the abdominal wall depends on its structure, in particular the collagen organization. The linea alba has a low tensile strength and is prone to fascial defects and hernias [1], whereas the rectus sheath and lateral abdominal muscles provide superior mechanical support, reducing the risk of recurrence [9,10,13].

Type I collagen, predominant in healthy tissues, provides mechanical strength, whereas the more elastic but weaker type III collagen favors scar fragility [14,15]. Increased activity of matrix metalloproteinases (MMP-2, MMP-9) involved in collagen degradation may weaken the abdominal wall and increase the risk of hernia recurrence [14,15,16].

1.4. Biochemical properties of collagen and its metabolism

Collagen, the main structural protein of the extracellular matrix, is essential for the stability of the abdominal wall. The balance between synthesis and degradation influences scar quality and the risk of recurrence.

A low collagen I/III ratio compromises connective tissue integrity, favoring recurrence [14,15]. Excessive matrix metalloproteinase activity accelerates collagen degradation, weakening the abdominal wall [14,15,16].

Genetic factors, such as COL1A1 and COL3A1 mutations, predispose to tissue fragility and increased risk of recurrence [17]. Diabetes, smoking and chronic inflammation also contribute to collagen degradation and impaired healing [18].

1.5. Pathogenesis of incisional hernias and risk factors

Recurrent incisional hernias occur due to impaired fascial healing process, leading to weakening of the abdominal wall.

Risk factors include ineffective surgical techniques, poor mesh fixation and abnormalities in collagen composition [19]. Obesity and diabetes also contribute to increased intra-abdominal pressure and altered collagen quality, which increases the risk of recurrence

[20,21]. Smoking impairs tissue vascularization, compromising the healing process and increasing the rate of recurrence [20,21].

1.6. Modern surgical techniques in hernia repair

The use of synthetic mesh is considered the gold standard in the treatment of incisional hernias, due to superior mechanical strength and lower recurrence rates compared to primary suture [22].

Laparoscopic techniques have proven effective in reducing postoperative infections and accelerating recovery [23]. On the other hand, robotic-assisted surgery improves the precision of the intervention, but its long-term efficacy requires further clinical validation [24].

The success of the repair depends not only on the surgical technique but also on the biological quality of the host tissue, in particular the collagen composition and the remodeling process of the extracellular matrix [14,15].

ORIGINAL PART-PERSONAL CONTRIBUTIONS

3. Working hypothesis and general objectives

This study proposes to investigate collagen disorders as a key factor in incisional hernia recurrence, with the central hypothesis that patients with recurrence have a low type I/III collagen ratio, leading to weaker scar tissue and reduced mechanical strength of the abdominal wall. The use of polarized light microscopy aims to assess the organization and maturity of collagen fibers, providing an advanced method of analysis over conventional histological techniques.

This approach can provide surgeons with critical information about connective tissue quality, helping to identify patients at high risk of recurrence and optimize surgical strategies. In addition, certain systemic conditions (such as aortic aneurysm) and patient-specific factors (diabetes, smoking, previous surgery) are suspected to influence collagen remodeling and contribute to tissue fragilization.

3.1. General objectives of the thesis

1. Evaluation of collagen composition in recurrent incisional hernias
 - To compare the collagen type I/III ratio between patients with recurrent hernias and a control group.
 - Analysis of collagen fiber orientation and quality using polarized light microscopy.
2. Identification of risk factors for hernia recurrence
 - Examination of demographic, biological and clinical factors influencing recurrence.
 - Investigate the role of connective tissue disorders (aortic aneurysm, varicose veins) in collagen metabolism.
 - Correlation of patient characteristics (diabetes, smoking, surgical history) with collagen abnormalities.
3. Development of a predictive model for hernia recurrence
 - Application of logistic regression analysis to evaluate the influence of collagen ratio on recurrence rates.

4. Contribution to future research on the pathophysiology of herniation pathophysiology and improvement of surgical outcomes.

- Provide evidence-based data on the role of collagen metabolism in hernia recurrence.
- Create a scientific basis for the development of innovative biological therapies to improve collagen synthesis and the healing process.

3.2. Expected impact of the study

A better understanding of the molecular mechanisms involved in the recurrence of incisional hernia, with the potential to improve prevention methods.

- Validation of polarized light microscopy as an advanced collagen assessment method applicable in surgery and histology.
- Development of a predictive model to identify patients at high risk of recurrence, facilitating a personalized approach in hernia surgery.
- Prospects for novel therapies, including collagen-targeted interventions, with the aim of improving abdominal wall healing and reducing recurrence rates.

By integrating these aspects, the study aims to optimize the management of recurrent incisional hernias, improving patients' quality of life and providing new therapeutic directions based on connective tissue biology.

4. General research methodology

This four-year (2020-2023) prospective observational study, carried out in the Surgery Clinic of the Nephrology Hospital "Dr. Carol Davila" in Bucharest, aims to evaluate the role of collagen remodeling in the recurrence of incisional hernias. The study focuses on the analysis of the collagen type I/III ratio and on the organization of collagen fibers in patients with recurrent hernia compared to a control group.

4.1. Study design and patient selection

The study includes two main groups:

- The recurrent hernia group (100 patients), consisting of individuals who required reintervention surgery at least two years after the first operation. All patients were initially operated with polypropylene mesh.

- The control group (30 patients) consisted of individuals who had been previously operated on for parietal defects using polypropylene mesh, but who did not have hernial recurrence and had undergone abdominal surgery for other pathologies.

Inclusion criteria: age ≥ 18 years, surgery with polypropylene mesh, absence of recurrence in the control group, and availability of tissues for analysis.

Exclusion criteria: Patients with active infections, autoimmune diseases, cancers, genetic connective tissue disorders (Ehlers-Danlos syndrome, Marfan syndrome) or who refused tissue sampling.

4.1.2. Surgical techniques used

All patients in the recurrence group previously underwent standardized hernia repair procedures using polypropylene mesh using two main techniques:

- On-Lay (Chevrel) technique - used in 70% of cases.
- Sub-Lay technique (Rives-Stoppa) - used in 30% of cases.

Surgeries were performed by a specialized team according to the 5-7 cm overlap standards to ensure methodological uniformity.

4.1.3. Tissue sample collection and histologic analysis

Tissues were harvested from the area of the recurrent hernia defect as well as from fascial tissue and alloplastic material of control patients. The minimum sample size was 1 cm², ensuring adequate histologic and immunohistochemical analysis.

4.1.4 Immunohistochemical staining for type I and III collagen

Immunohistochemical staining was used for the evaluation of type I and III collagen, and a subgroup of 10 patients (5 from each group) was selected for this analysis due to the high cost.

4.1.5. Polarized light microscopy for collagen analysis

Polarized light microscopy was used to provide a detailed view of collagen fibers, identifying differences between mature (type I) and immature (type III) collagen.

The technique allows analysis of fiber orientation, alignment and density, providing a clear insight into collagen remodeling abnormalities. The study utilized the Olympus SZX2-ILLT stereomicroscope, equipped with a high-resolution imaging system, avoiding structural alteration of tissues.

Image analysis was performed with open-source software: ImageJ (FIJI) for pre-processing, CT-FIRE for collagen segmentation, QuPath for mesh-tissue interaction, OrientationJ for birefringence and MorphoLibJ for structural weakness detection.

4.1.6. Statistical analysis methods

The study utilized Microsoft Excel, Epi-Info 7, and SPSS Statistics 21.0 to analyze demographics, parietal defect size, herniation distribution, and correlation of collagen I/III ratio with recurrence risk.

Statistical methods applied included Kaplan-Meier estimation, Log-rank test, Chi-square test and logistic regression to identify risk factors. Correlation analysis assessed the relationship between collagen disorganization and hernial recurrence.

1. RESULTS

5.1. Demographic study

5.1.1 Sex distribution and its possible implications

The study showed a higher prevalence of males in both groups (57% in the recurrent hernia group, 52% in the control group), suggesting biological, mechanical and behavioral factors in recurrence. A low collagen I/III ratio in men may weaken the abdominal wall, and strenuous physical activities increase mechanical stress. Estrogen offers women a protective effect, but their risk remains high due to postpartum muscle weakening, obesity and gynecologic surgery

5.1.2 Age distribution and its significance

The incidence of hernia recurrence is higher in patients aged 50-60 years, with an increased number of cases in men. As they age, patients show collagen degradation and decreased tissue strength. Women are more present in younger and middle age groups due to gynecologic factors and history of abdominal surgery.

5.2 Parietal defect size analysis

Defect size influences the risk of recurrence in the recurrent group:

- 20% of patients had defects ≤ 5 cm,
- 55% 6-10 cm defects,
- 25% ≥ 10 cm defects.

Large defects (>10 cm) indicate an increased risk of surgical failure, and recurrence even in small defects suggests connective tissue weakness associated with collagen abnormalities.

5.3. Recurrence rank analysis

- 83% had a grade II recurrence (a previous surgery),
- 17% had a grade III recurrence (two failed repairs).

These data suggest that deficient collagen remodeling is a primary cause of recurrence, rather than simple technical failure.

5.4. Topographic localization

Defects predominated in the midline, with 32% supraumbilical, 28% periumbilical, 18% subumbilical, 13% hypogastric, and 9% in the flanks. Structural weakness of the linea alba favors recurrence.

5.5 Analysis of risk factors in the development of incisional hernia

5.5.1 Pre-operative factors

Metabolic and systemic factors influence collagen remodeling and the risk of recurrence. Diabetes (30% vs. 20%) delays healing, and hypertension and ischemic heart disease affect tissue perfusion. Obesity and chronic obstructive bronchopneumopathy increase intra-abdominal pressure, favoring recurrence. Collagen disorders (aneurysms, varices) were exclusive in the recurrent group, suggesting a tissue defect. Multiple prior surgeries significantly increase the risk of recurrence, the most common being gynecologic (26%), urologic (14%) and colorectal (13%). Aortic aneurysm (9%) indicates a possible collagen dysfunction, a major biological factor in parietal repair failure.

5.5.2 Intraoperative Factors

Incision type and impact on recurrence

The majority of patients with recurrent hernia had median (78%) compared to pararectal (9%) and transverse Pfannenstiel (13%) incisions. The median incision is associated with a higher risk of recurrence due to the natural weakness of the white line.

Surgical techniques and risk of recurrence

In the recurrence group, two main techniques were used:

Recurrence occurred regardless of the technique used: On-lay (70%) exposes the mesh to tension and Sub-lay (30%) provides better integration. This suggests that collagen abnormalities influence recurrence more than mechanical factors.

5.5.3 Postoperative Factors

Postoperative complications, more common in recurrence, include seroma (27%), infection (13%) and hematoma (11%), affecting collagen remodeling. Increased intra-abdominal pressure (coughing, constipation, vomiting) aggravates scar tissue vulnerability.

5.6. Contributions on the surgical treatment of recurrent incisional hernias

5.6.1. Pre-operative preparation

Surgical treatment of recurrent incisional hernias requires a complex approach, which starts with rigorous preoperative preparation. This includes metabolic and nutritional optimization, diabetes control, anemia correction, and respiratory preparation to minimize postoperative complications. Advanced imaging such as CT is essential for defect assessment and intervention planning.

5.6.2. Type of anesthesia used

General anesthesia was used in all patients to ensure optimal muscle relaxation, facilitating hernia sac reduction and prosthetic mesh placement. The choice of the type of anesthesia was influenced by the size and complexity of the hernia, preventing hemodynamic instability and respiratory complications.

5.6.3. Surgical treatment of recurrent incisional hernias

Laparoscopy was used in 84% of cases because of its benefits such as reduced pain, shorter hospitalization (3 vs. 5.8 days) and rapid recovery. Conversion to open surgery was necessary in 4% of cases due to adhesions. For complex hernias, the mesh was placed intraperitoneally for optimal integration.

Complications were more common in open surgery, with higher rates of seroma, hematoma and respiratory failure. Operative time was shorter in laparoscopy (1.5 vs. 2.5 hours). Mortality was low (1%) and infections were effectively prevented.

Pain assessment through Visual Analogue Scale (VAS) showed lower levels in laparoscopy, confirming the minimally invasive advantages. Up to 1 year postoperatively, no

recurrences were reported, but long-term follow-up is necessary. These data support laparoscopy as the optimal approach for recurrent incisional hernias.

5.6. Macroscopic evaluation of non-integrated mesh in hernia recurrence

Mesh integration into the host tissue is essential for successful incisional hernia repair. However, in some cases, the mesh does not incorporate adequately, which favors recurrence. Macroscopic examination of unintegrated meshes during surgical reinterventions provides essential information about the mechanisms leading to repair failure.

The main findings include:

- Lack of tissue integration, where nets remain mobile or encapsulated in collagen-poor fibrotic tissue.
- Mesh displacement, wrinkling and shrinkage, caused by inadequate fixation or excessive fibrotic response, which compromises the stability of the repair.
- Chronic inflammation and seroma formation, indicating a prolonged response to the foreign body, affecting mesh integration.

Analysis of patients operated open (20 cases) showed that 70% of the examined meshes were non-integrated, suggesting a major role of poor collagen quality in repair failure. These findings support the hypothesis that disturbances in collagen metabolism contribute to hernia recurrence, affecting the integration of prosthetic materials and the stability of the repair.

5.7.1. Comparative histopathologic analysis of the two groups of patients

Histologic analysis of the scar tissue in patients with recurrent incisional hernia revealed significant structural differences compared to patients in the control group. These differences are essential for understanding the pathologic mechanisms that contribute to the recurrence of abdominal wall defects, indicating that recurrence is not solely determined by surgical technique but also by the quality of connective tissue.

Changes in collagen structure in patients with recurrent hernia

In the recurrent hernia group, a marked disorganization of collagen fibers was observed, which appear fragmented and chaotically distributed. This misalignment compromises the

mechanical integrity of the scar, reducing its resistance to abdominal wall stresses. In addition, the excessive fibrosis, instead of reinforcing the tissue structure, forms a dense but non-functional tissue that fails to effectively support the healing process. Persistent chronic inflammatory infiltrates and inadequate vascularization were evident in the majority of the cases analysed, contributing to defective regeneration of the extracellular matrix and poor integration of scar tissue.

Collagen structure in the control group

The control group, without hernia recurrence, showed well-organized collagen with aligned and densely distributed fibers, providing scar stability. Balanced extracellular matrix deposition and adequate vascularization promoted efficient healing, reducing the risk of recurrence.

Limitations of the study include the high cost of immunohistochemical techniques and the degradation of alloplastic materials by toluene, affecting the analysis of mesh-tissue interaction. However, the data suggest that imbalance of collagen remodeling and poor connective tissue quality are more important factors in hernia recurrence than surgical technique.

5.8. Immunohistochemical Analysis

Immunohistochemical analysis of collagen was used to evaluate the structure and organization of scar tissue in patients with recurrent incisional hernia and in the control group. An optimal balance between type I and III collagen is essential for scar maturation and mechanical stability of the abdominal wall.

In the recurrent hernia group, a low collagen I/III ratio (0.82) was observed, with a low proportion of type I collagen (45%) and an increased level of type III collagen (55%). This composition indicates poorly organized scar tissue, prone to mechanical failure and recurrence. In contrast, the control group showed a collagen I/III ratio of 3.00, characterized by a high level of type I collagen (75%) and a low level of type III collagen (25%), suggesting a well matured and stable scar.

These results confirm that defective collagen remodeling is a key factor in hernial recurrence. Reduced expression of type I collagen and persistent type III collagen compromises

the mechanical stability of scars, increasing the risk of recurrence. Thus, optimizing the extracellular matrix remodeling process could be an important strategy to prevent hernia recurrence.

5.9.1. Stereomicroscopic analysis

Stereomicroscopy has been used as a method to assess collagen quality in patients with recurrent incisional hernia, having the advantage of a superior three-dimensional visualization of connective tissue architecture and its interaction with biomaterials used in the repair of parietal defects. Unlike conventional microscopy, which requires treatment with solvents such as Toluene - with the risk of dissolving the mesh and compromising histological analysis - stereomicroscopy offers a non-invasive method with a greater depth of field, allowing the observation of the distribution of collagen fibers and their organization around the prosthetic material.

This technique was applied in the analysis of tissues from two groups of patients: the recurrent hernia group, where hernia repair failed, and the control group, without recurrence. The results demonstrated significant differences in collagen architecture, mechanical stability of the scar, and degree of mesh integration.

5.9.3 Results of stereomicroscopic analysis

1. Collagen fiber organization and integrity

In the recurrent herniated group, collagen fibers appeared fragmented, disorganized and poorly oriented with numerous discontinuities in the extracellular matrix. This irregular collagen structure suggests poor mechanical stability, which predisposes connective tissue to dehiscence and hernia recurrence. Mesh integration was poor, with signs of excessive fibrosis and a tendency toward irregular encapsulation. Adipocytic infiltration was also more pronounced, which may signal an atypical tissue response, with insufficient fibroblast proliferation and limited connective tissue regeneration capacity.

In the control group, collagen fibers were densely packed, parallel oriented and integrated into a coherent structural network. This organization allowed a uniform distribution of mechanical stresses and greater resistance to intra-abdominal forces, thus preventing

recurrence. Mesh integration was optimal with no signs of chronic inflammation or biomaterial rejection.

2. Mesh-tissue interaction and the healing process

A key aspect highlighted by stereomicroscopy was the interaction between the mesh used for hernia repair and the host tissue. In the recurrent hernia group, poor integration of the mesh was observed, with an incompletely formed fibrous network around the biomaterial. This phenomenon was associated with a lack of efficient fibroblast colonization and a predominance of lipocytes, suggesting a prolonged inflammatory response and an altered healing process. Poor fixation of the mesh in the connective matrix was accompanied by increased fragmentation of collagen fibers, increasing the risk of biomaterial mobilization and mechanical failure of repair.

In the control group, stereomicroscopic analysis showed optimal integration of the mesh, with increased fibroblast density and balanced organization of the extracellular matrix. This allowed for a controlled healing response with uniform collagen deposition and adequate vascularization, which supported stable fixation of the biomaterial in the host tissue and contributed to the prevention of hernia recurrence.

3. Collagen birefringence and connective tissue maturity

Using polarized light microscopy, significantly lower birefringence was observed in the recurrent hernia group, indicating a predominance of type III collagen, a marker of connective tissue immaturity. The more elastic and loosely organized type III collagen is present in the initial stages of healing, but should be progressively replaced by type I collagen to form a mature and stable scar. In this group, the persistence of immature collagen indicated defective tissue remodeling and an increased susceptibility to recurrence.

In the control group, high birefringence demonstrated a well matured collagen network with a predominance of type I collagen. This organization gave the tissue superior tensile strength and increased biomechanical stability, reducing the risk of surgical repair failure.

4. Indicators of structural weakness and risk of recurrence

The final collagen analysis revealed a discontinuous extracellular matrix and poor mesh integration in the recurrent hernia group, compromising the biomechanical strength of the scar

tissue and predisposing to recurrence. Disorganization of collagen fibers and predominance of immature collagen resulted in fragile scars unable to withstand intra-abdominal stress.

In contrast, the control group showed a well-structured tissue architecture with a dense collagen network and a balanced mechanical stress distribution, reducing the risk of dehiscence. These results confirm that defective collagen remodeling and insufficient mesh integration are key factors in hernia recurrence, highlighting the need for strategies to optimize tissue regeneration.

6. Discussion

Recurrence of incisional herniation remains a major problem in abdominal wall surgery, with recurrence rates varying depending on patient characteristics, the surgical technique used, and natural healing processes [25]. Our study emphasizes the impact of collagen metabolism on hernia recurrence, demonstrating that not only the surgical technique influences the success of repair, but also the structural quality of collagen and inflammatory factors.

6.1 Gender and age-related factors in incisional hernia recurrence

The study showed a higher recurrence in males (57%) than females (43%), attributed to hormonal and structural differences. Males have a low collagen I/III ratio, weakening the abdominal wall (26), and testosterone inhibits collagen synthesis [26]. In contrast, estrogen stimulates healing [27]. Intense physical activity increases the risk of recurrence in men, and in women, pregnancy and gynecologic interventions affect the abdominal wall. The most affected group was the 50-60 age group due to collagen degradation and associated comorbidities [28].

6.2 Biological factors influencing hernia recurrence

Patients with recurrent hernia have significant imbalances in collagen metabolism, with less efficient fibroblasts and a genetic predisposition for poor healing [14]. Increased levels of hyperglycemia and cholesterol affect collagen stability, while chronic inflammation degrades newly formed collagen, impairing scar remodeling.

6.3. Comorbidities and their impact on hernia recurrence

Comorbidities such as diabetes (30%), hypertension (53%) and COPD contribute to recurrence by impairing tissue perfusion and increasing intra-abdominal pressure. Collagen disorders, such as aortic aneurysms and varicose veins, have also been identified exclusively in patients with recurrence, suggesting underlying tissue fragility.

6.4. Social factors: Role of smoking and obesity in hernia recurrence

Smoking, identified in 73% of recurrent cases, inhibits collagen I synthesis and stimulates matrix metalloproteinases, favoring tissue degradation [29]. Obesity, present in 54% of patients with recurrence, increases the mechanical stress on the abdominal wall and favors chronic inflammation, impairing healing processes [29].

6.5 Postoperative factors influencing wound healing and their impact on hernia recurrence

Postoperative complications such as seromas (27%), hematomas (11%) and infections (13%) affect collagen remodeling and increase the risk of recurrence. Seromas indicate persistent inflammation, hematomas suggest vascular fragility, and infections cause collagen degradation, affecting scar strength.

6.6. Surgical strategies and mesh positioning

The laparoscopic approach was used in 84% of cases, significantly reducing postoperative complications and recovery time. Conversion to open surgery was necessary in only 4% of cases because of extensive adhesions [30]. Correct placement of the prosthetic material in a different anatomic plane from the previous repair optimized tissue integration and stabilized the repair.

6.7 Histopathologic examination of tissue and collagen remodeling in recurrent incisional hernias

Histopathological analysis revealed significant collagen disorganization and defective extracellular matrix (ECM) remodeling in recurrent hernias. A lower type I/III collagen ratio, excessive but ineffective fibrosis, and chronic inflammation compromised scar integrity. Weak neovascularization further weakened tissue stability. In contrast, the control group showed well-structured collagen, balanced ECM deposition, minimal inflammation, and better vascularization, contributing to stronger scar formation.

6.8. Stereomicroscopic analysis of collagen and its role in hernia recurrence

Stereomicroscopic evaluation revealed significant differences in collagen organization between patients with recurrent incisional hernia and the control group. In recurrent cases, collagen fibers are fragmented, disorganized, and poorly integrated with the mesh, indicating defective remodeling of the extracellular matrix. Fibrotic encapsulation rather than functional integration further weakened tissue stability. Decreased birefringence and increased adipocyte infiltration suggested persistent inflammation and poor fibroblast activity, preventing adequate tissue repair.

In contrast, the control group showed well-structured collagen with a strong birefringence, indicating a predominance of mature type I collagen. Better fibroblast proliferation and balanced extracellular matrix remodeling contributed to a more stable scar and superior mechanical strength. These findings confirm that defective collagen remodeling and poor mesh integration play a key role in hernia recurrence, highlighting the need for improved therapeutic strategies.

7. Conclusions and personal contributions

7. Conclusions and Personal Contributions

7.1. Conclusions

1. Study Objectives – This doctoral research has successfully achieved its proposed objectives, providing a detailed analysis of collagen remodeling in recurrent incisional hernias and highlighting its implications for surgical outcomes.
2. Correlation Between Collagen Deficiencies and Hernia Recurrence – The study demonstrated a significant association between structural abnormalities of collagen and the increased risk of hernia recurrence, emphasizing the importance of the biological quality of tissue in the long-term success of hernia repair.
3. Imbalance of the Collagen I/III Ratio – Stereomicroscopic, histopathological, and immunohistochemical analyses revealed a reduced collagen I/III ratio in patients with recurrent incisional hernias, indicating a predominance of immature collagen with lower mechanical resistance.
4. Relevance of Stereomicroscopy in Collagen Analysis – The use of polarized light microscopy and advanced stereomicroscopy techniques enabled a detailed three-dimensional visualization of collagen architecture, facilitating a better understanding of fiber orientation and mechanical load distribution in hernia-affected tissues.
5. Limitations of Immunohistochemistry as a Standard Method – The high costs of immunohistochemical analysis, coupled with the need for specialized monoclonal antibodies, restricted the applicability of this method to a limited subgroup of patients. This highlights the advantages of stereomicroscopy as an accessible and feasible alternative in routine practice.

6. Limitations of the Postoperative Follow-up Period – The study was limited to a one-year postoperative monitoring period, underscoring the need for extended longitudinal studies to assess progressive collagen remodeling and the risk of late recurrence.

Future Research Directions

Optimizing Reconstruction Strategies in Hernia Surgery – Expanding research using polarized light microscopy to analyze the structure and orientation of collagen fibers based on the depth of prosthetic implantation is recommended.

Validation of the Hypothesis on Deep Prosthesis Placement – Future studies should evaluate the impact of prosthesis positioning on tissue integration and repair durability through quantitative analysis of collagen birefringence and extracellular matrix remodeling at different implantation depths. This approach could provide fundamental data for developing evidence-based recommendations to optimize surgical techniques and reduce the risk of recurrence.

7.2. Personal contributions

A major innovation of this study was the introduction of stereomicroscopy as an optimal method to assess mesh-tissue integration and collagen remodeling in recurrent hernias. In contrast to traditional histopathological techniques, stereomicroscopy offers a three-dimensional perspective, providing a detailed structural assessment of collagen fiber organization and mesh colonization.

This technique has proven superior in identifying structural abnormalities, such as collagen disorganization, excessive fibrosis, and poor fibroblast integration, which contribute to hernia recurrence. In addition, stereomicroscopy is a cost-effective and non-destructive alternative to immunohistochemistry, avoiding expensive monoclonal antibodies and specialized laboratory infrastructure.

The study contributed significantly by:

1. Improved visualization of mesh-tissue interaction and fibroblast integration.
2. Providing qualitative and quantitative assessments of collagen fiber structure and mechanical integrity.

3. Demonstrate the advantages of a high-resolution, artifact-free examination technique.
4. Validate stereomicroscopy as a superior and cost-effective alternative to conventional histopathologic and immunohistochemical evaluations.

By integrating stereomicroscopy with advanced digital image analysis, the study introduced a novel and reproducible methodology for quantifying collagen defects and understanding their role in herniation recurrence. These findings pave the way for future research on tissue biomechanics, personalized surgical approaches, and improved mesh designs for long-term hernia repair success.

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